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(54) Title: PYROPHEOPHORBIDES AND THEIR USE IN PHOTODYNAMIC THERAPY

(57) Abstract

Pyropheophorbide compounds are injected into a host and accumulate in tumor tissue to a higher degree than surrounding normal tissues. When the pyropheophorbide compounds are exposed to a particular wavelength of light the compounds become cytotoxic and destroy the tumor or diseased tissue without causing irreversible normal tissue damage. The pyropheophorbide compounds have shown improved results as compared to drugs currently used in photodynamic therapy. Further, they absorb light further in the red, optimizing tissue penetration and are retained in the skin for short time periods relative to other drugs used in photodynamic therapy.

PYROPHEOPHORBIDES AND THEIR USE IN PHOTODYNAMIC THERAPY

Cross-References

This application is a continuation-in-part of our earlier filed application Serial No. 07/597,786 filed October 15, 1990 which is a continuation of application Serial No. 07/221,804 filed July 20, 1988 which is now U.S. Patent 5,002,962 issued March 26, 1991 both of which are incorporated herein by reference and to which we claim priority under 35 USC §120.

FIELD OF THE INVENTION

This invention relates generally to photosensitive therapeutic compounds and photodynamic therapy (PDT). More particularly, the invention relates to pyropheophorbides, formulations that contain such and their use in the treatment of cancer.

BACKGROUND OF THE INVENTION

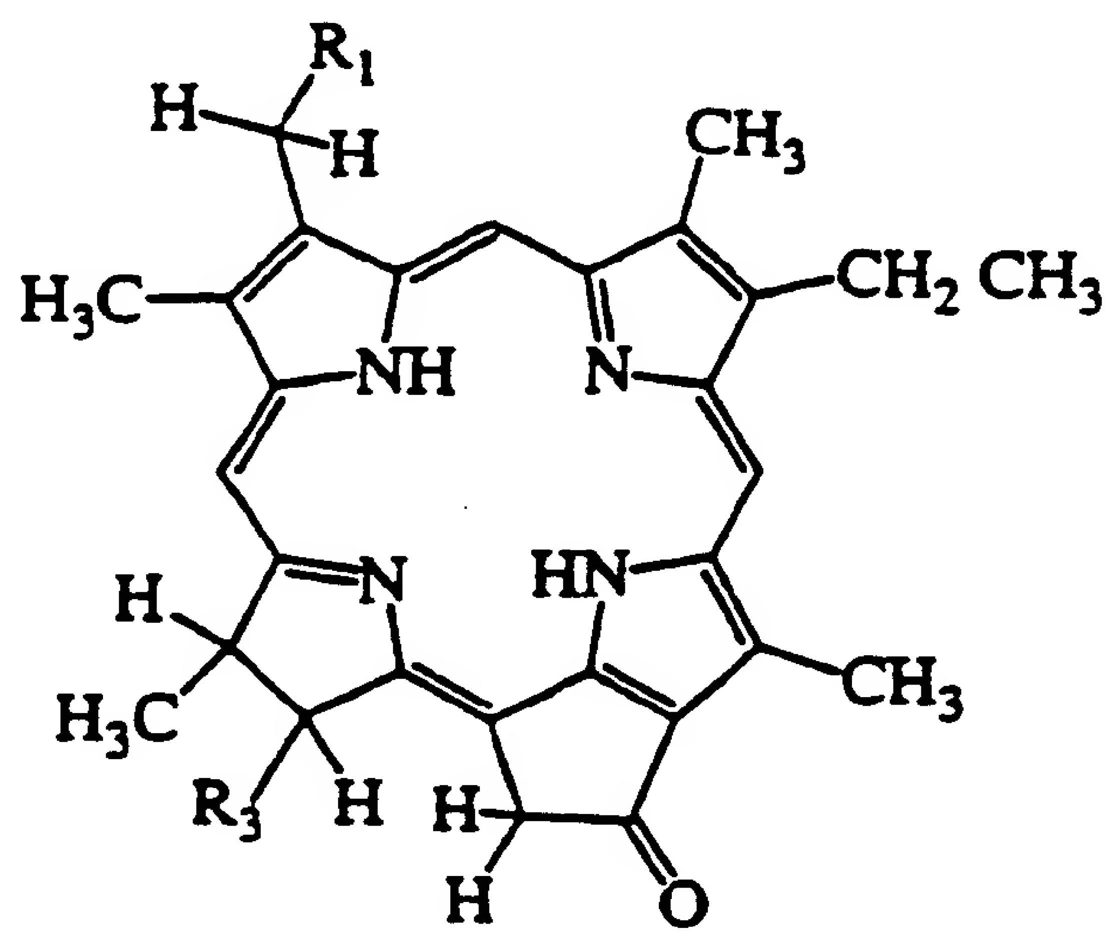
As described in U.S. Patent 5,002,962, porphyrin related compounds accumulate at higher concentrations in tumor tissue as compared to normal tissue, and that irradiation of these compounds using light of the proper wavelength results in an energized form which, upon decay, results in cytotoxicity. It is believed that excitation of the porphyrin or related material results in the formation of singlet oxygen which is in fact the toxic agent. However, the compounds administered apparently do not degrade in this process.

5,002,962 are useful in photodynamic therapy as are compounds and methods disclosed in U.S. Patents 4,920,143 and 4,883,790.

SUMMARY OF THE INVENTION

Pyropheophorbide compounds and pharmaceutical compositions containing such compounds can be used in methods of photodynamic therapy. The pyropheophorbides are encompassed by the following general structural formula I or II.

I



wherein R_1 is CH_2OR_2 where R_2 is a primary or secondary alkyl containing 1 to 20 carbons; and R_3 is $-CO_2R_4$ where R_4 is H or an alkyl containing 1 to 20 carbons. Other compounds of the invention are encompassed by formula II as follows:

conducting photodynamic therapy using the conjugates and their compositions.

A primary object of the invention is to provide pyropheophorbide compounds, pharmaceutical compositions containing such compounds and method of treatment carried out using such compounds in a photodynamic therapy.

Other objects are to provide methods of treating humans with tumor cells which cells replicate abnormally fast, treating atherosclerosis or inactivating bacteria or virus infections.

A feature of the present invention is that the pyropheophorbide compounds of the invention absorb light further into the red portion of the spectrum as compared with conventional compounds used in photodynamic therapy.

An advantage of the present invention is that the pyropheophorbide compounds and pharmaceutical compositions of the invention optimize tissue penetration and are retained in the skin for relatively short periods of time as compared with other compounds used in photodynamic therapy.

Another advantage of the present invention is that the pyropheophorbide compounds of the invention have a greater toxicity with respect to tumor cells and diseased tissue as compared with the toxicity of conventional compounds used in photodynamic therapy.

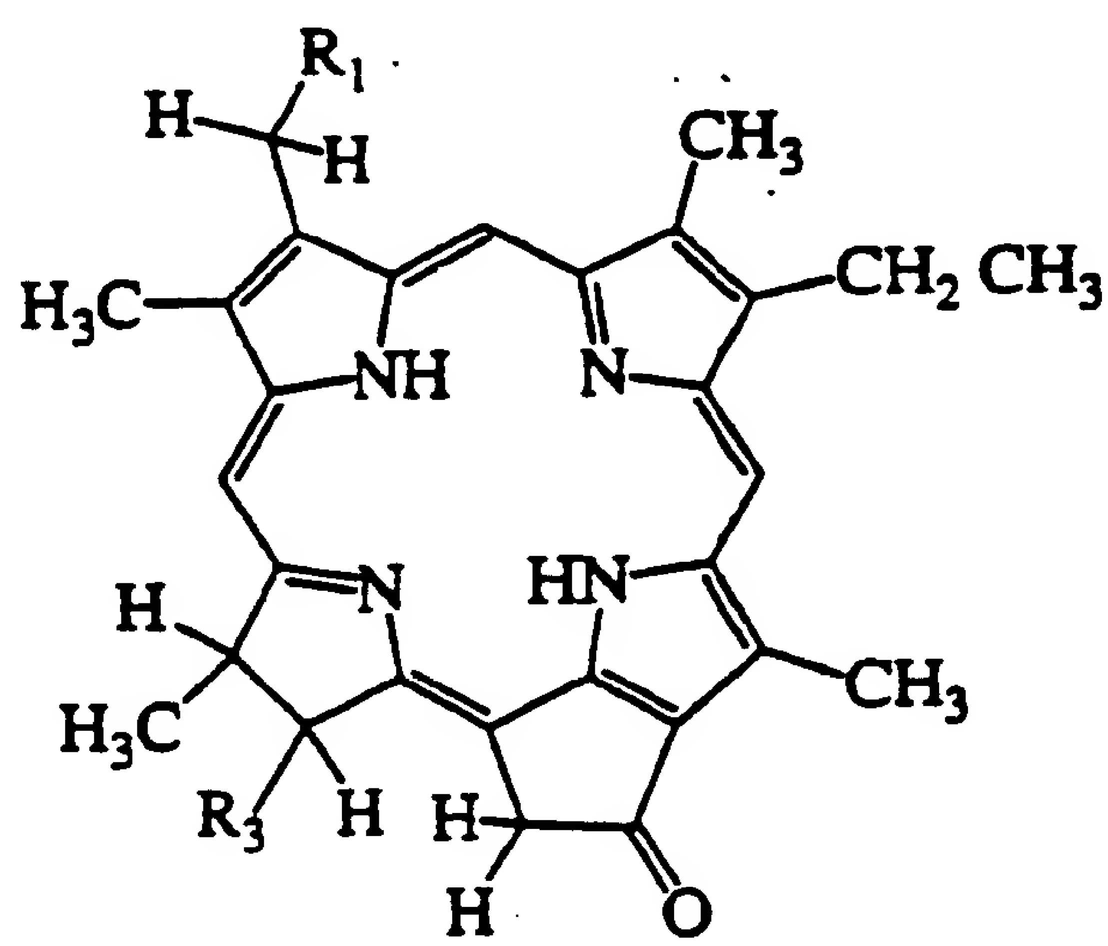
Another advantage of the invention is that the pyropheophorbides can be synthesized as free acids (e.g. in formula I or II when R_3 or R_7 are $-CO_2H$) allowing ease in formulation without the need for liposomes or detergents.

Another advantage of the invention is the pyropheophorbide of the invention are active at very low doses of injected material as compared to conventional photosensitizers used in photodynamic therapy.

Unless defined otherwise all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art of photodynamic therapy. Although any methods and materials similar or equivalent to those described herein may be used in the practice or testing of the present invention, attempts have been made to describe preferred methods and materials below.

The essence of the invention is the disclosure of novel compounds and pharmaceutical compositions containing such compounds which have been found to be highly effective in the treatment of cancer when used in connection with a photodynamic therapy. More specifically, the compounds are pyropheophorbide compounds which are encompassed by the following general structural formulae I and II.

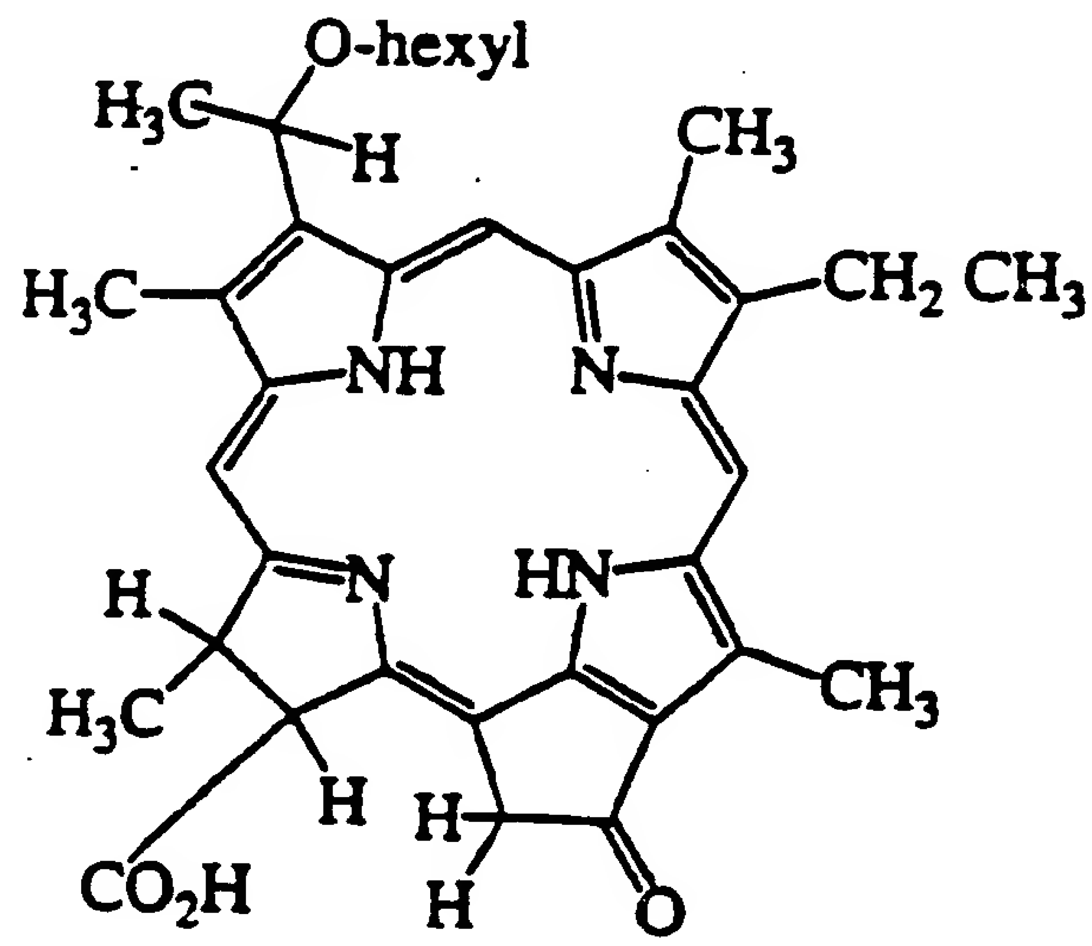
I



wherein R_1 is CH_2OR_2 where R_2 is a primary or secondary alkyl containing 1 to 20 (preferably 5-20) carbons; and R_3 is $-CO_2R_4$ where R_4 is H or an alkyl containing 1 to 20

particularly effective in the treatment of cancer when used in connection with photodynamic therapy. Structural formula IIa is put forth below:

IIa



A generalized reaction scheme for the synthesis of the compound of structural formula IIa is put forth below:

Starting Materials

The starting material for preparation of the red light-absorbing compounds is methyl pheophorbide-a, which is isolated from Spirulina destridratada by the method of Smith and Goff (D. Goff, Ph.D. Thesis, Univ. of Calif., Davis, CA 95616, 1984 incorporated herein by reference). Briefly, 500 gm dried Spirulina was slurried in a large volume of acetone and then liquid nitrogen was added to form a frozen slush. The slush was transferred to a 3-necked, 5-liter round bottom flask and heated to reflux under nitrogen with stirring for 2 hours. The mixture was filtered through Whatman paper on a Buchner funnel with extensive acetone washing. The extraction and filtration process was repeated 2 more times; all green color could not be removed from the solid.

The green filtrate was evaporated and purified by flash chromatography on Grade V neutral Alumina, eluting first with n-hexane to remove a fast running yellow band and then with dichloromethane to obtain the major blue/gray peak containing pheophytin-a. Treatment of pheophytin-a with 500 ml sulfuric acid in methanol for 12 hours at room temperature in the dark under nitrogen was followed by dilution with dichloromethane. The reaction mixture was rinsed with water and then 10% aqueous sodium bicarbonate and the organic layer was dried, evaporated, and the residue recrystallized from dichloromethane/methanol to obtain 1.8 gm methyl pheophorbide-a. Methyl pheophorbide-a appears to be inactive in the in vivo tumorcidal activity assay when injected at a dose of 5 mg/kg.

Conjugates and Labeled Pyropheophorbides

In addition to using compositions which consist essentially of the above-defined compounds or preparations as active ingredient, it is possible to use

prepared as described by Malcolm, A., et al, Ex Hematol (1984) 12:539-547; polyclonal or monoclonal preparations of anti-M1 antibody as described by New, D., et al, J Immunol (1983) 130:1473-1477 (supra) and B16G antibody which is prepared as described by Maier, T., et al, J Immunol (1983) 131:1843; Steele, J.K., et al, Cell Immunol (1984) 90:303 all of which publications are incorporated herein by reference.

The foregoing list is exemplary and certainly not limiting; once the target tissue is known, antibody specific for this tissue may be prepared by conventional means. Therefore the invention is applicable to effecting toxicity against any desired target.

The ligand specific for receptor refers to a moiety which binds a receptor at cell surfaces, and thus contains contours and charge patterns which are complementary to those of the receptor. It is well understood that a wide variety of cell types have specific receptors designed to bind hormones, growth factors, or neurotransmitters. However, while these embodiments of ligands specific for receptor are known and understood, the phrase "ligand specific for receptor," as used herein, refers to any substance, natural or synthetic, which binds specifically to a receptor.

Examples of such ligands include the steroid hormones, such as progesterone, estrogens, androgens, and the adrenal cortical hormones; growth factors, such as epidermal growth factor, nerve growth factor, fibroblast growth factor, and so forth; other protein hormones, such as human growth hormone, parathyroid hormone, and so forth; and neurotransmitters, such as acetylcholine, serotonin, and dopamine. Any analog of these substances which succeeds in binding to the receptor is also included.

conjugation does not form part of the invention. Therefore, any effective technique known in the art to produce such conjugates falls within the scope of the invention, and the linker moiety is accordingly broadly defined only as being either a covalent bond or any linker moiety available in the art or derivable therefrom using standard techniques.

The compounds of the invention per se or the conjugates may be further derivatized to a compound or ion which labels the drug. A wide variety of labeling moieties can be used, including radioisotopes and fluorescent labels. Radioisotope labeling is preferred, as it can be readily detected <u>in vivo</u>.

The compounds which are alone or are conjugates with a specific binding substance can be labeled with radioisotopes by coordination of a suitable radioactive cation in the porphyrin system. Useful cations include technetium and indium. In the conjugates, the specific binding substances can also be linked to label.

<u>Administration and Use</u>

In general, the pyropheophorbide compounds of the invention are administered to a host such as a human suffering from cancer in therapeutically effective amounts by any suitable means such as injection which may be IV or IM or may be administered transdermally. The pyropheophorbide compounds of the invention accumulate in tumor cells to a much higher degree than they accumulate in surrounding normal tissues. After being provided with sufficient time so as to accumulate in the tumor tissue, the pyropheophorbide compounds are exposed to a particular wavelength of light which causes the compounds to become cytotoxic, thus destroying the tumor or diseased tissue which the pyropheophorbide compounds have accumulated in. This is accomplished without causing

pharmaceutical compositions may be found, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pennsylvania, latest edition. The compositions, labeled or unlabeled, can be administered systemically, in particular by injection, or can be used topically.

Injection may be intravenous, subcutaneous, intramuscular, or even intraperitoneal. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid form suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol and the like. Of course, these compositions may also contain minor amounts of nontoxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and so forth.

Systemic administration can also be implemented through implantation of a slow release or sustained release system, by suppository, or, if properly formulated, orally. Formulations for these modes of administration are well known in the art, and a summary of such methods may be found, for example, in Remington's Pharmaceutical Sciences (supra).

If the treatment is to be localized, such as for the treatment of superficial tumors or skin disorders, the compositions may be topically administered using standard topical compositions involving lotions, suspensions, or pastes.

The quantity of compound to be administered depends on the choice of active ingredient, the condition to be treated, the mode of administration, the individual subject, and the judgment of the practitioner. Depending on the specificity of the preparation, smaller or larger doses may be needed. For compositions which are highly specific to target tissue, such as those which comprise conjugates with a highly specific monoclonal

EXAMPLES

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to make the pyropheophorbide compounds and pharmaceutical compositions of the invention and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.), but some experimental errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in degrees Centigrade, and pressure is at or near atmospheric.

EXAMPLE 1

Methyl pyropheophorbide-a (2): Methyl pheophorbide-a (1, 1.0 g) was obtained from alga Spirulina destridratada by following the procedure described in K.M. Smith, D.A. Goff and D.J. Simpson, J. Am. Chem. Soc., 1985, 107, 4941-4954; and R.K. Pandey, D.A. Bellnier, K.M. Smith and T.J. Dougherty, Photochem. Photobiol., 1991, 53, 65-72, both of which are incorporated herein by reference. The methyl pheophorbide-a was heated under reflux in collidine (100 ml) for 90 min during slow passage of a stream of nitrogen. See G.W. Kenner, S.W. McCombie and K.M. Smith, J. Chem. Soc. Perkin Trans. 1973, 1, 2517-2523, incorporated herein by reference. The solution is evaporated (0.1 mm Hg) and the residue was recrystallized from dichloromethane/methanol. Yield 820 mg; 91%, m.p. 217-219°C, lit. 220-225°C; H. Fisher and A. Stern, Die Chemie des Pyrrole, vol II, Part 2, pp. 64 and 74,

mm Hg) and the resulting 1-bromo ethyl derivative was immediately treated with n-hexanol (3.0 ml) under nitrogen atmosphere. The reaction mixture was stirred at room temperature for 45 min, diluted with dichloromethane (100 ml). The dichloromethane layer was washed with water (3X200 ml) till the aqueous phase is neutral and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a residue, which was chromatographed over Alumina Grade III (6% water/neutral Alumina) and eluted with dichloromethane. The first fraction was a mixture of the starting material and the desired product (minor quantity). Further elution with same solvent gave the desired product. The appropriate eluates were combined. Evaporation of the solvent afforded a sticky solid, which can be crystallized from dichloromethane/ hexane. Yield 70%. (see Scheme-1), Vis,(max); 408 (90 000); 471 (3 200), 506 (8600); 536 (8,500); 604 (7,250); 660 (41 500). NMR, ppm; 9.79, 9.51, 8.53 (each s, 1H, meso H); 5.90 (q, 2H, —CH (O-hexyl)CH_3; 5.08-5.30 (q, 2H, 10 - CH_2); 4.47 (m, 8H); 4.29 (m, 7-HO; 3.75 (q, 2H, CH_2CH_3); 3.67 (s, 3H, $CH_2CH_2CO_2CH_3$), 3.67 (s, 6H, 2 X CH_3); 3.38 and 3.27 (each s, CH_3); 2,68 (7a-H) 2.28 (7a'-H), 2.55 (7b-H); 2.20 (7b'-H); 1.80 (d, 3H, CH_2CH_3); -1.70 (s, 2H, 2 NH); for the hexyl group, 3.72 (t, 2H, O-CH_2CH_2); 1.73 (2H, CH_2); 1.25 [bs, merged, 6H, $(CH_2)_3$]; 0.78 (t, 3H, CH_3). (see fig. 1).

2-{1(O-hexyl)ethyl}devinyl pyropheophorbide-a (5): Pyropheophorbide-a (3, 200 mg) was reacted with 30% HBr/acetic acid and then with n-hexanol by following the method as discussed for 4 and the desired product was isolated in 60 to 65% yield. The structure was confirmed by NMR spectroscopy.

approximately 24 h, the hind foot of the animal is exposed to the same dose of either laser light at 660-670 nm (135 Joules/cm^2) or the Xenon arc lamp (283 Joules/cm^2) as above. The reaction of the foot is scored for damage over the next few days to determine the maximum effect, which in this case is a value 0.3 equivalent to slight edema. If the internal between the injection and light treatment is extended to approximately 48 h, the foot reaction is zero (no damage incurred), indicating either clearance or metabolism of the sensitizer.

Data obtained as a result of experiment carried out is put forth below in Table 1.

The data put forth in Table 1 clearly demonstrates that the pyropheophorbide compounds of the invention are activated by light having a wavelength of about 660 nm. Further, when the compound were administered by injection and subjected to light having a wavelength of about 660 nm, the treatment was found to be highly effective with respect to reducing tumor size in as little as seven days.

Further, the data of Table 1 show compounds of the invention clear skin over a period of 24-48 hours after administration. This is a desirable feature in that the patient is not subjected to prolonged cutaneous photosensitivity. The data of Table 1 also show that the hexyl ethers of formula II are preferred over methyl ethers in terms of effecting tumor growth when used in photodynamic therapy.

While the present invention has been described with reference to specific compounds, formulations and methods, it is to be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the true spirit and scope of the invention. In addition, many modifications may be made to adapt to a particular individual, method of administration, process of synthesizing, etc., which are within the scope of the present invention. All such modifications are intended to be within the scope of the claims appended hereto.

4. A pharmaceutical composition useful in treatment of a target virus, cells or tissue, comprising:

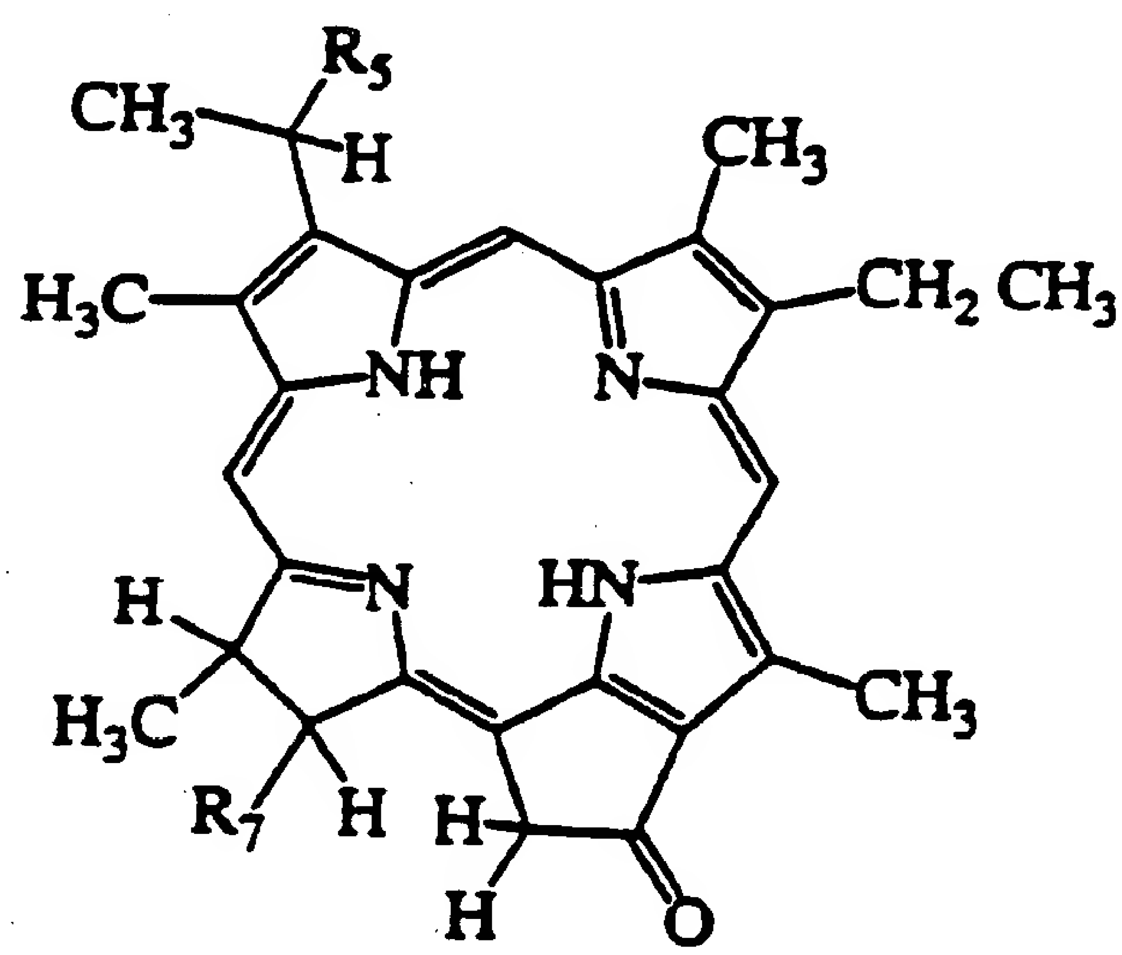
an effective amount of the compound of claim 1 in admixture with a pharmaceutically acceptable excipient.

5. A conjugate which consists essentially of the compound of claim 1 covalently bound to a target-specific component selected from the group consisting of an immunoglobulin and a receptor ligand.

6. A pharmaceutical composition useful for labeling malignant tissue which comprises the compound of claim 1 associated with a label.

7. A compound of formula II:

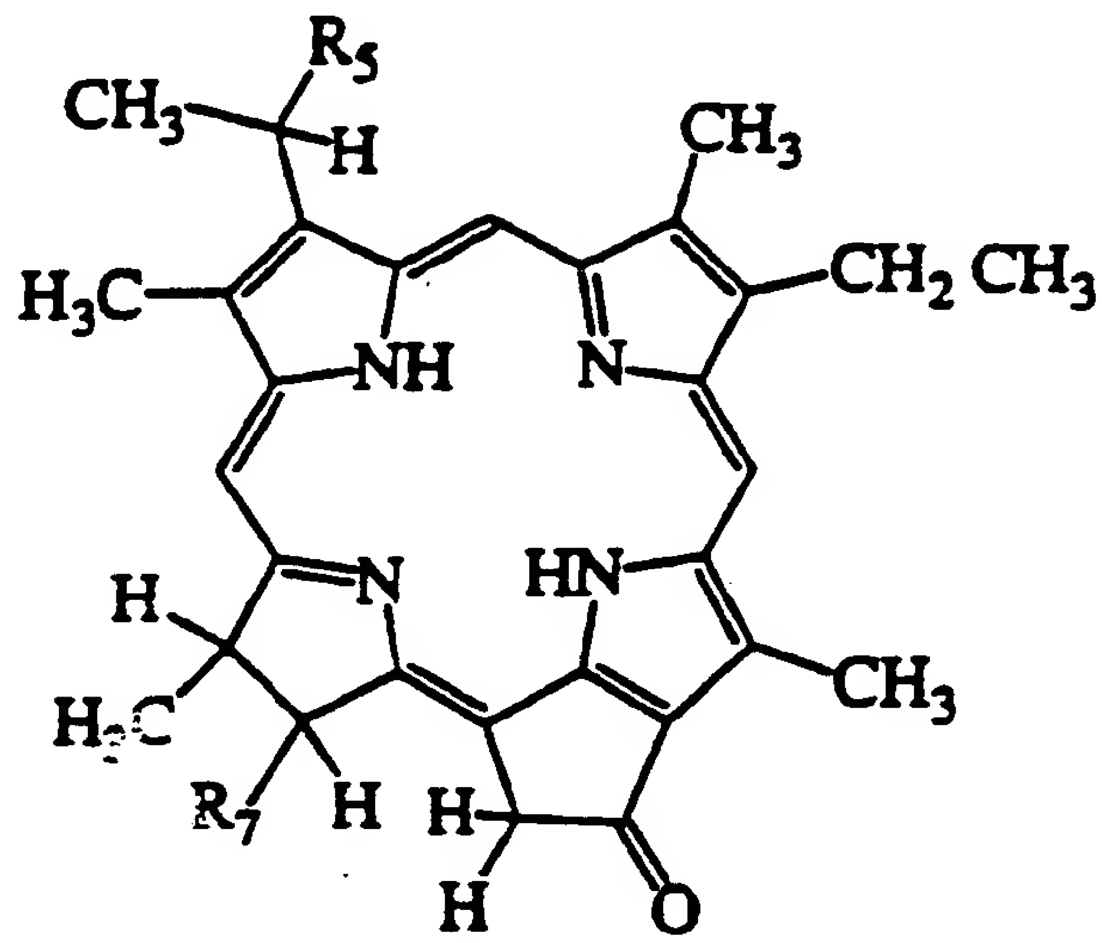
II



wherein R_5 is $-OR_6$ where R_6 is a primary or secondary alkyl containing 1 to 20 carbons and R_7 is $-CO_2R_8$ where R_8 is H or an alkyl containing 1 to 20 carbons.

administering to the human a therapeutically effective amount of a compound of formula II

II



wherein R_5 is OR_6 where R_6 is a primary or secondary alkyl containing 5 to 20 carbons and R_7 is $-CO_2R_8$ where R_8 is H or $-CH_3$;

allowing the compound of formula I to accumulate on the abnormal cells; and

irradiating the compound of formula I with a wavelength of light which is absorbed by the compound of formula I and thereby generating a cytotoxic effect with respect to the abnormal cells.

13. The method as claimed in claim 12 wherein the compound is administered in an amount in the range of 0.01 mg/kg to 1.0 mg/kg of body weight and is administered at timed intervals in the range of from every 3 hours to every 72 hours for over a period of from 1 day to 30 days and the wavelength of the light is in the range of 600 to 700 nm.

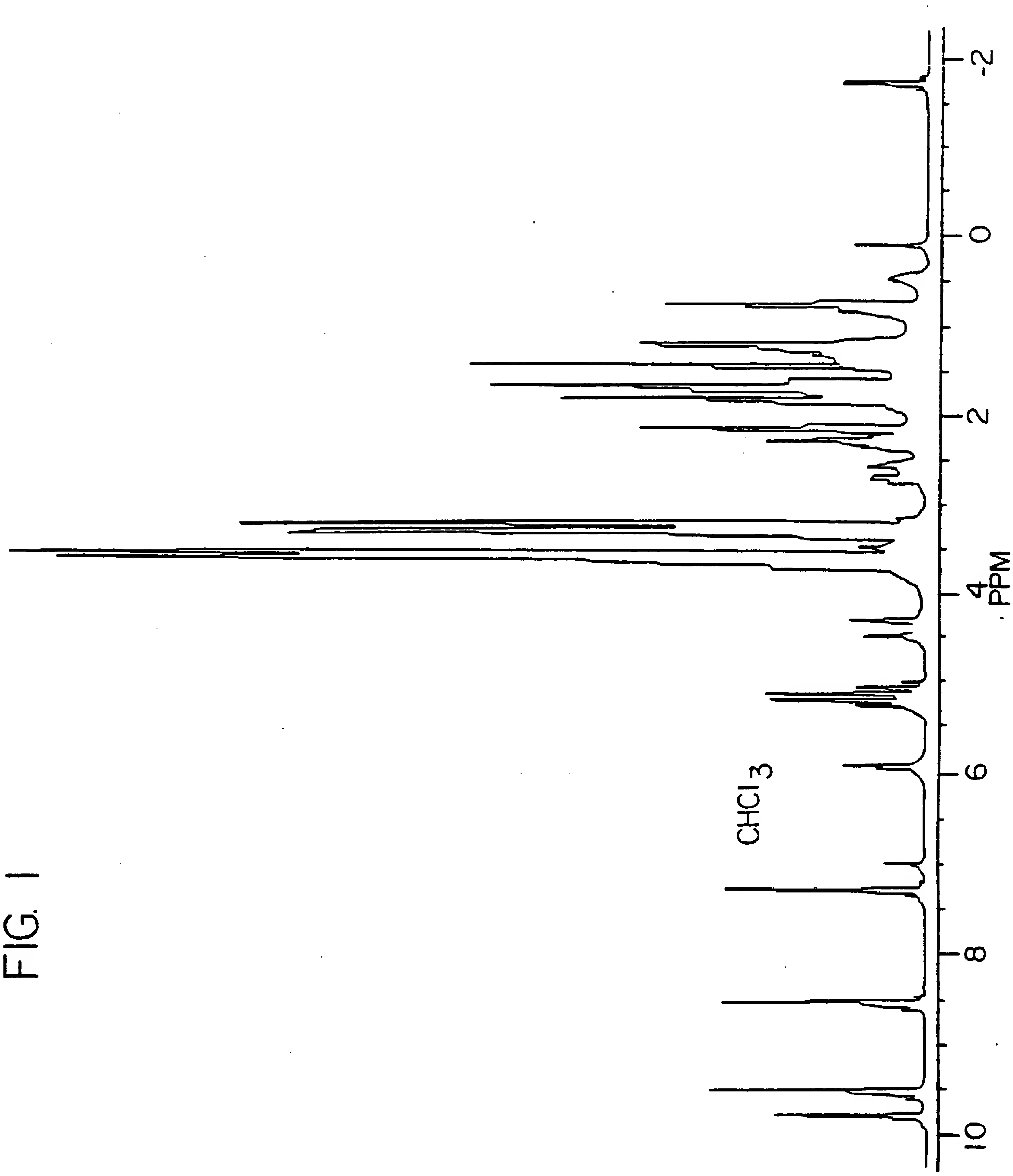


FIG. 1

FIG 1